Understanding the Biology of Bone Metastases: Key to the Effective Treatment of Prostate Cancer

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Abstract
Advanced prostate cancer often metastasizes to distant sites, most commonly to the bone (1). Although the mortality rate associated with prostate cancer has been declining over the last decade, metastases substantially shorten survival time. Developing effective therapies for prostate cancer bone metastases has been challenging. Thus, the current goals are to uncover the mechanism that drives prostate cancer progression to bone and to use this knowledge to develop biomarkers for detecting metastases early and selecting targets for therapy.

Our approach toward these goals comprises three steps. First, we framed our research direction so as to allow us to address a clinical dilemma. Second, we used multiple clinically relevant model systems to test our hypotheses. Third, we followed up on these laboratory findings with early validation in human tissues. This step has allowed us to understand the potential relevance of the research findings to human disease and to prioritize the research direction and clinical trials efficiently. We believe that this approach will increase the prospects of obtaining clinically meaningful insights.

Clinical Characteristics of Prostate Cancer
Prostate cancer is unique because it has a very restricted clinical progression path. Tumors that initiate in the prostate frequently metastasize to bone, where tumor cells seem to exert an effect that is characterized by a very distinct clinical pattern, i.e., bone-forming metastasis. This characteristic of prostate cancer bone metastases suggests that unique interactions occur between prostate cancer cells and the bone microenvironment. Although this phenomenon has long been known, the nature of these interactions and the way in which they affect the progression of prostate cancer in bone are unknown. Consequently, how to interfere with those interactions and whether such interference would result in a favorable clinical outcome remain in question.

Focusing on Bone as a Clinically Relevant Target
We hypothesized that interrupting the specific interactions between prostate cancer cells and bone would be an effective therapy for prostate cancer bone metastasis. We first tested the clinical relevance of this hypothesis. Based on the results of the Trans-Canada clinical trial, which has shown that a bone-targeting radiopharmaceutical, strontium 89, could alter the course of the disease (2), we did a phase II randomized clinical trial (3, 4). Our patients who responded to chemotherapy and received strontium 89 survived longer than those given only chemotherapy (Fig. 1; refs. 3, 4). These findings provided the impetus to focus on bone as a clinically relevant target and supported the notion that therapies targeting both the bone and prostate cancer compartments are beneficial for treating prostate cancer bone metastasis. We then reasoned that if we could elucidate the underlying mechanisms and identify corresponding markers, we could also develop targeted...
therapies for bone metastases. Clinical trials and laboratory investigations aiming to understand such interactions were carried out in parallel.

**Developing Clinically Relevant Models for Mechanistic Studies**

Obtaining samples of metastatic prostatic carcinoma tissues is extremely difficult. Bone metastases from prostate cancer are typically osteoblastic; therefore performing biopsies of these dense tissues is difficult and done only during therapeutic intervention. Although the use of circulating tumor cells isolated from blood is feasible, those cells are no longer in contact with the stromal components of the bone and may not provide insights into the underlying biology of the microenvironment. To obtain clinically relevant specimens for laboratory studies, we sampled bone tissues by using transilial biopsy, a procedure widely used by hematologic oncologists. With this procedure, which we did routinely with morbidity acceptable to patients, we could obtain clinically relevant metastatic tissues and bone marrow supernatants for exploring the proteomic profiles of prostate cancer bone metastases.

We also developed in vitro and in vivo model systems that may reflect aspects of human prostate cancer bone metastases. Few cell lines or xenograft models reflect the complex steps that lead to prostate cancer metastasis and progression in bone. Thus, it has been difficult to study the mechanisms of the migration of cancer cells to the bone and their subsequent progression. Our strategy to overcome this limitation was to generate a panel of prostate cancer cell lines and xenografts in mice, using tissues derived from patients at different stages of prostate cancer progression. We have succeeded in generating two cell lines and two xenografts from bone metastasis specimens thus far. The bone-derived cell lines, MDA-PCa-2a and MDA-PCa-2b, exhibit many aspects of prostate cancer in bone: androgen regulation, bone formation, and prostate-specific antigen expression (5). One of our two new xenograft models exhibits characteristics seen in patients with prostate cancer in bone, e.g., androgen-receptor negativity, prostate-specific antigen negativity, and bone formation. The other model represents another type of prostate cancer in bone in that the xenograft can proliferate in bone and is androgen-receptor positive but induces neither bone formation or bone destruction. These and other models being generated have given us insights into the heterogeneous nature of prostate cancer bone metastases at the molecular level and will be invaluable for discovering the mechanisms behind prostate cancer progression in bone.

**Discovery in the Laboratory: Interactions of Prostate Cancer Cells with the Bone Microenvironment**

Paracrine factors likely mediate the interactions between prostate cancer cells and bone. Bone marrow supernatants from patients with or without bone metastases are being used as a discovery platform on which we apply proteomics discovery technique to screen for paracrine factors implicated in prostate cancer progression in bone. The functional relevance of these paracrine factors in conferring pathologic progression will be tested in our bone-derived cell lines and xenografts. This approach is exemplified in the study of p45-ErbB3, a soluble

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<sup>3</sup> Li et al. manuscript submitted.
<sup>4</sup> N.M. Navone, unpublished data.
form of ErbB3 identified in the bone marrow of patients with bone metastasis (6, 7). In parallel, we are characterizing paracrine factors secreted by the cell lines and xenografts. The strategy of using clinically relevant samples and models in our investigation will accelerate the translation of our findings into clinical practice.

**Application to the Practice of Oncology**

Although laboratory investigations into the mechanism of prostate cancer progression in bone are ongoing, we have evaluated two bone-targeting therapeutic strategies clinically. Platelet-derived growth factor (PDGF) is one of the paracrine factors secreted by prostate cancer cells and has been shown to have effects on osteoblasts (8) and endothelial cells in the bone microenvironment (9). Activation of PDGF receptors in prostate cancer bone metastases has been observed in animal models of human prostate cancer (10). In an animal model, inhibition of PDGF receptor tyrosine kinase activity by using imatinib could lead to inhibition of prostate cancer growth in bone (11), possibly by inhibiting angiogenesis. Therefore, testing whether imatinib combined with docetaxel would improve the survival of patients with prostate cancer progression in bone was reasonable.

As predicted, we found that bone-remodeling markers were modulated in patients treated with imatinib in the docetaxel-imatinib randomized clinical trial (12), supporting the hypotheses that blocking PDGF can modulate the bone microenvironment in prostate cancer bone metastasis. Because docetaxel-imatinib therapy failed to improve the progression-free or overall survival of the patients (12), we hypothesize that the effect of imatinib on the bone microenvironment is insufficient to alter the progression of prostate cancer in bone. Many explanations for this are possible, including the heterogeneous nature of the disease and the involvement of multiple paracrine factors. In a recent animal model study, blockage of PDGF receptor activity by imatinib led to increased expression of vascular endothelial growth factor, an angiogenesis factor (13). These observations have led to the hypothesis that targeting multiple tyrosine kinases is required for treating patients with advanced cancer. A clinical trial using an agent that blocks PDGF and vascular endothelial growth factor receptor tyrosine kinase activities for the treatment of bone metastasis is being undertaken to test this hypothesis.

We and others have speculated that the prostate and the bone microenvironment may have shared properties that account for the tropism of the bone for prostate cancer. Studying human prostate cancer bone metastases is limited by the difficulty of gaining access to sufficient tissues for study, but this is not a problem with localized high-grade prostate cancer. We reasoned that preoperative studies on patients with high-grade prostate cancer would be feasible and that this approach may lead to the identification of determinants for human prostate cancer progression in bone. We have tested this hypothesis by using thalidomide, which reportedly increases the progression-free and overall survival of patients with advanced castration-resistant prostate cancer when used in combination with docetaxel (14). In _in vitro_ studies, thalidomide did not inhibit cancer cell growth (15), suggesting that the therapeutic effect of thalidomide results from

Fig. 2. Shared signaling pathways implicated in the tumor microenvironment of high-risk prostate cancer and bone.
modification of the tumor microenvironment rather than the tumor cells themselves. To test this, we did a preoperative study in which patients with high-risk prostate cancer were treated with thalidomide followed by radical prostatectomy. When the prostate specimens were examined, we found attenuated sonic hedgehog pathway signaling, as reflected in decreases in the transmembrane protein Smoothened and in expression of the transcription factor gli2 (16). Because sonic hedgehog signaling governs epithelial-mesenchymal cross-talk during organogenesis, including prostate development (17), these observations are consistent with the hypothesis that the therapeutic benefit of thalidomide in advanced prostate cancer is attributable to its interrupting of the interactions between prostate cancer cells and the surrounding stromal components. These findings also suggest that the sonic hedgehog signaling pathway is another therapeutic target for further consideration. Because we found the preoperative approach informative and clinically feasible, this approach may be used to screen for strategies for treating bone metastases, in which obtaining metastatic prostatic carcinoma tissues is extremely difficult.

Conclusion

By conducting clinical and laboratory investigations in parallel in our translational oncology practice, we and others have identified new mechanisms underlying prostate tumorigenesis (Fig. 2). Our studies have implicated specific molecular pathways in the stromal-epithelial interaction in bone. These mechanistic insights led us to search for determinants of bone metastasis development to improve our prediction of further disease progression. They also led to a new approach to managing prostate cancer, which is to focus on blocking the stromal-epithelial interaction. Identifying the pathways mediating these interactions will be key to achieving effective therapy for prostate cancer bone metastases.

References

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